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(54) Title: PHARMACEUTICAL COMPOSITION THAT MAKES CELLS EXPRESSING MHC CLASS II ANTIGENS TARGETS FOR CYTOTOXIC T-CELLS

(57) Abstract

The present invention concerns a pharmaceutical composition for treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens. The composition comprises a substance making said cells targets for cytotoxic T cells. The substance may be selected from the group consisting of staphylococcal enterotoxines, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.

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PHARMACEUTICAL COMPOSITION THAT MAKES CELLS EXPRESSING MHC CLASS II ANTIGENS TARGETS FOR CYTOTOXIC T-CELLS.

The present invention concerns a new agent and a new method for the activation of T cells. Specifically the invention concerns a new agent and method for treating certain cancer forms and autoimmune processes.

Background of the invention

MHC antigens or transplantation antigens, which in man are also called HLA antigens, play an essential part in T cell activation and the concomitant immune response. Thus e.g. protein antigens are dependent on interactions with MHC antigens for presentation to and activation of T cells during a normal immune response. Likewise, MHC dependent presentation of autoantigens are of key importance in T cell dependent autoimmune processes.

A certain type of MHC antigens, i.e. the so called MHC class II antigens are expressed by a variety of normal cells either constitutionally or after activation (1). Among tumour cells, malignancies of the haematopoietic system commonly express MHC class II molecules. Although MHC class II antigens are not restricted to tumour cells, they may constitute an attractive target for therapy since most normal cells would be spared.

A potent agent capable of eliminating MHC class II antigen expressing cells may therefore be useful both for interruption of autoimmune processes and for the destruction of certain malignant cells.

Summary of invention

Accordingly, the present invention concerns a method of treating malignancies involving cells expressing MHC class II antigens, said method comprising administering to a living body a composition including a substance making said cells targets for cytotoxic T cells.

The invention also comprises a pharmaceutical composition for treating these malignancies. The composition includes a substance making said cells targets for cytotoxic T cells.

The substance should have the ability of activating the cytotoxic T cells, redirect them to MHC class II antigens irrespective of nominal specificity and thereby inactivate or lyse the MHC class II expressing cells.

According to the invention it has been found that a preferred substance is of bacterial origin and has the ability to direct T cells to lyse the target MHC class II antigen expressing cell. It is believed that the substance should interact with parts of the T cell receptor complex (TCR) and, more specifically, particular sequences in the T cell receptor V beta chains. Preferably the substance or bacterial structure is selected from the group comprising of staphylococcal enterotoxines, SE:s, such as SEA, SEB, SEC, SEC1, SEC2, SEC3, SED or SEE, toxoids active fragments or peptides of these enterotoxins and substances having essentially the same mode of action, such as other bacterial products from staphylococcal strains e.g. Toxic shock syndrome toxins (TSST-1) and bacterial exoproteins produced from various other strains, e.g. streptococcal exoproteins and proteins produced by mycoplasma arthritidis, which have similar capacity to interact with certain TCR V-beta sequences. Genetically or chemically modified substances based on the above mentioned structures could also be used.

An example of a substance which does not fall within the scope of the invention is protein A. This protein emanates from Staphylococcus but it is both structurally and functionally different from the staphylococcal enterotoxins.

Staphylococcus enterotoxins (SE), which are produced by Grampositive bacteria are a family of molecules of protein structure, that are commonly known for causing food poisoning (2). They are also potent T cell mitogens causing T cell activation and concomitant production of lymphokines (3-5). However, SE unlike most mitogens are strictly dependent on the presence of accessory cells expressing MHC class II molecules (6,7).

Moreover, strong circumstantial evidence has been presented that SEA directly interacts with certain T cell receptor families during their activation of lymphocytes (8,9).

It has been previously suggested (WO-A1-89/09619) to use SEA for the treatment of cancer. According to this publication, however, SEA is used for the treatment of blood in vitro (=outside the patients body), and the SEA is removed before the activated cells are reinfused to the patient, whereas the discovery on which the present invention is based discloses that SEA and functionally similar agents can act as bifunctional crosslinkers in vitro. Activating and bringing T cells to tumour cells in the body can be of potential use for the in vivo treatment of certain types of cancer and autoimmune diseases.

The agent according to the present invention is preferably in the form of a pharmaceutical composition comprising the substance, together with a pharmaceutically acceptable vehicle or carrier.

The composition is furthermore preferably administered parenterally, and should thus include those adjuvants which are commonly used within this field. Thus the enterotoxin or fragment thereof could be administered in the form of lipid emulsions, or combined with human serum albumin or mixtures of amino acids. The amount of the active component of the composition can vary within broad ranges e.g. 1 ng - 100 mg.

In addition to systemic administration it is also possible to use the composition according to the invention for local administration. The composition could e.g. be locally administered in an arthritic joint.

Conditions which may be treated by the compositions according to the invention are variuous forms of autoimmune diseases and tumor diseases e.g. lymphomas and leukemias.

The invention is further illustrated by the following examples which should not be construed as limiting the invention.

Examples

To determine Staphylococcal enterotoxin directed cell mediated cytotoxicity (SDCC) we employed a panel of human anti-HLA-A2 cytotoxic T cell lines as effector cells and the HLA-$A2^-DR^+$ EBV transformed RAJI B-cell line, the R.2.2 HLA-DR^- mutant of the RAJI cell line and the HLA-$A2^+DR^+$ EBV transformed BSM B-cell line. The anti-HLA-A2 specific T cell lines were established from primary MLC cultures and repeated restimulations with mitomycin-treated HLA-$A2^+$ stimulator cells and recombinant IL-2 (20 units/ml). These T cell lines strongly lysed the specific HLA-A2 expressing target but not irrelevant targets. The effect of Staphyloccal enterotoxin (SE) A, B, C1 and D was studied using SE from Tox Tech (Madison, WI., USA). To demonstrate that HLA-DR is the target molecule in SDCC we used the above described panel of target cells and monoclonal antibodies (mAb) directed to HLA-DR, MHC Class II, W6/32 and CD 23.

Chromium labeling and incubation of the target cells with SEA:

$0.75x10^6$ target cells and 150 µCi ^{51}chromium (Amersham Corp., Arlington Hights, England) were incubated for 45 minutes at 37°C in a volume of 100 µl. The cells were kept in complete medium containing RPMI-1640 medium (Gibco, Paisley, GBR) supplemented with 2.8 % (v/v) 7,5 % $NaHCO_3$, 1 % sodium pyrovate, 2 % 200 mM L-glutamine, 1 % 1M Hepes, 1 % 10mg/ml gentamicin and 10 % fetal calf serum (FCS, Gibco, Paisley, GBR). After the incubation the cells were washed once in complete medium without FCS and incubated 60 minutes at 37°C and washed and resuspended in complete medium containing 10 % FCS: $5x10^3$ target cells were added to each well of U-bottom 96-well microtiter plates (Costar, Cambridge, USA).

Cytotoxicity assay

The effector cells were added to the wells at various effector/target cell ratios. The final volume in each well was 200 µl. Each test was done in triplicate. The plates were incubated 4 hours at 37°C after which the released chromium was harvested using SCS Harvesting frames (Skatron, Norway). The amount ^{51}Cr was determined in a gamma-counter (Cobra Auto-gamma, Packard). The percentage cytotoxicity was computed by the formula %cytotoxicity = (X-M)/(T-M) * 100, were X is the chromium release as cpm obtained in the test sample, M is the spontaneous chromium release of target cells incubated with medium, and T is the total chromium release obtained by incubating the target cells with 1% sodium dodecyl sulfate.

Results

Human allospecific T cell lines, which demonstrated exquisite specificity for HLA-A2 showed strong cytotoxicity against the irrelevant HLA-$A2^-DR^+$ RAJI target cell and increased cytotoxicity against the specific HLA-$A2^+DR^+$ BSM target cell, when SEA was added in the assay (Fig. 1 A-B). The T cell mediated SE directed cellular cytotoxicity (SDCC) occured at very low concentrations of SE, with maximal effect at 10-0.1 ng/ml and half maximal effect was seen at about 0.01 ng/ml (Fig. 1 C). The SDCC phenomenon could be induced by several of the SE. The 5.2 T cell line showed selective reactivity towards SEA, whereas the 5.1 and 11.2 lines reacted with several SE (Fig. 2 A-C). Activation of T cells by the combination of SEA and SEB resulted in an additive increase in cytotoxicity to the RAJI target cell (Fig. 2 C). Blocking studies with monoclonal antibodies (mAb) directed to different cell surface structures demonstrated that the mAb G8, which has recently been shown to interact with a SEA binding site on the HLA-DR molecule, strongly inhibited the SDCC (Table 1. Expt #1). In contrast, the HB 96 mAb, which interacts with a monomorphic MHC Class II determinant, unrelated to the SEA binding epitope and the MHC

Class I and CD23 mAb did not show any inhibitory activity (Table 1. Expt.#1). Preincubation experiments with SE demonstrated that the binding of SE to the RAJI cells and not to the T cell line was a prerequisite for the induction of SDCC against the RAJI cells (Table 1. Expt.#2). The MHC Class II negative RJ2.2.5 mutant of the RAJI cell line was found to be completely resistent to SDCC, while the Class II expressing parental cell line was an excellent target (Table 1. Expt.#3). These observerations implicates that HLA-DR is the main target molecule in SDCC.

Legend to Figure 1. SEA induced T cell mediated cytotoxicity against RAJI and BSM target cells. The cytotoxicity of an anti-HLA-A2 allospecific T cell line to the RAJI ($HLA\text{-}A2^{-}DR^{+}$) and BSM ($HLA\text{-}A2^{+}DR^{+}$) lymphoblastoid cell lines in the presence or absence of 1 ng/ml SEA was examined at various effector/target ratios in a standard 4 hour ^{51}-chromium release assay and is expressed as % specific cytotoxicity. Figure 1 C demonstrates the dose-response of SEA at concentrations 10^{-5} to 10^{2} ng/ml. SEA was added directly into the microtiter wells together with ^{51}-Chromium labelled RAJI target cells and effector T cells at indicated effector/target cell ratios. Addition of SEA to the target cells in the absence of effector cells did not result in any significant change in the spontaneous release of ^{51}-chromium.

Legend to Figure 2. Induction of SDCC by SEA, SEB, SEC1 and SED. The cytotoxicity of the anti-HLA-A2 allospecific T cell lines 5.2, 5.1 and 11.2 against RAJI target cells in the presence or absence of the various SE at concentration 1 ng/ml was examined in a 4 hour ^{51}-chromium release assay.

Table 1. HLA-DR is the target molecule in SDCC

Effector	Target	Additive SE	Additive mAb	% Cytotox at E/T ratio[A] A	B	C
Expt.#1.						
5.2	RAJI	-	-	1	0	0
		+	-	30	19	14
		+	HB96	31	23	16
		+	G8	8	6	3
		+	W6/32	37	28	19
		+	CD23	40	27	19
Expt.#2.						
11.2	RAJI	-		2	0	0
		+		56	34	14
	RAJI/SE	-		33	28	12
11.2/SE	RAJI	-		2	0	0
Expt.#3.						
12.1	RAJI	-		3	2	3
		+		43	30	22
	R12.2.5	-		10	11	8
		+		12	9	6
	RAJI/SE	-		32	24	21
	R12.2.5/SE	-		12	9	7

[A] Cytotoxicity of anti-HLA-A2 allospecific T cell lines against HLA-DR$^+$ RAJI cells and MHC Class II negative Raji mutant R.2.2.5 cells. The different mAb were added at the initiation of the culture at an optimal concentration of 20 ug/ml purified ascites Ig (HB 96 and W6/32) or at a ascites dilution 1/300 (G8 and CD23). SE was added to the assay at 0.1 ng/ml (Expt.#1.), 10 ng/ml (expt. #2.) or 1 ng/ml (Expt.#3.). Preincubation of target and effector cells was performed with SE at 1 ng/ml (Expt.#2: RAJI/S and 11.2/SE) or 10 ng/ml (Expt. #3: RAJI/SE and R.2.2.5/SE) for minutes at room temperature. The cells were extensiveley washed

prior to use in the assay. Addition of SE to the different target cells did not result in any significant change in the spontaneous release of ^{51}-chromium. The effector/target (E/T) ratios were 20:1, 10:1 and 5:1 in Expt.#1 and #3 and 30:1, 10:1 and 3:1 in Expt.#2.

Composition for injections

1 ng - 100 mg SEA (available from Tox Tech Madison, W1) is dissolved in 0,1 - 5 ml PBS (Phosphate Buffered Saline) and optionally human serum albumin or another conventionally used vehicle.

Injections for administration are intravenous bolus injections including 1-5 ml or continuous infusion of 500 ml during a perioc of 1-24 h.

For demonstration of in vivo anti-tumor effects of SE the following two experimental approaches are performed:

1. 10^6 murine MHC II$^+$ A20 lymphoma cells in 0,3 ml Saline are injected subcutaneously in C57B1/6 mice. The mice are treate with 1 ug of SEA in 0,5 ml Saline intravenously or as contro Saline alone. The treatment is given as one dose every week. Tumor growth is followed every day by measurement of total tumor volume. After 4 weeks of therapy the mice are sacrificed.

2. 10^5 murine MHC II$^+$ transfected B 16 melanoma cells in 0,3 ml Saline are injected intravenously in C57B1/6 mice. The mice are treated with 1 ug of SEA in 0,5 ml Saline intravenously or as control Saline alone. The treatment is given as one dose every week. Mice are sacrificed after 3 and 4 weeks of therapy and the number of lung metastasis are evaluated.

REFERENCES

1. Guillemot, F., Auffrey, C., Orr, H.T., and Strominger, J.: MHC antigen genes In: Molecular Immunology (Harnes, B.D. and Glover, D.M. (Eds.)) pp 81-143; IRL Press, Oxford, 1988.

2. Bergdoll, M.S.: Enterotoxins In: Staphylococci and Staphyloccocal Infections Vol. II (Easman, C.S.F. and Adlam, C. (Eds.)) pp 559-598; Acad. Press, New York, 1983.

3. Langford, M.P., Stanton, G.J., Johnson, H.M.: Biological effects of Staphylococcal enterotoxin A on human peripheral blood lymphocytes, Infect. Immun. 22:62-8, 1978.

4. Carlsson, R. Sjögren, H.O.: Kinetics of IL-2 and interferon-gamma production, expression of IL-2 receptors, and cell proliferation in human mononuclear cells exposed to staphylococcal enterotoxin A. Cell. Immunol. 96:175-181, 1985.

5. Fisher, H., Dohlsten, M., Andersson, U., Hedlund, G., Ericsson, P-E., Hansson, J., Sjögren, H.O.: Production of TNF-a and TNF-b by staphylococcal enterotoxin A activated human T cells. J. Immunol. Methods, in press, 1989.

6. Fisher, H., Dohlsten, M., Lindvall, M., Sjögren, H.O., Carlsson, R.: Binding of staphylococcal enterotoxin A to HLA-DR on B cell lines. J. Immunol. 142:3151-3157, 1989.

7. Mollick, J.A., Cook, R.G., Rich, R.R.: Class II MHC molecules are specific receptors for staphylococus enterotoxin A. Science 244:817-820, 1989.

8. Lando, P.A., Dohlsten, M., Kalland, T., Sjögren, H.O., Carlsson, R.: The TCR-CD3 complex is required for activation of human lymphocytes with staphylococcal enterotoxin A. Scand. J. Immunol., in press, 1989.

9. White, J., Herman, A., Pullen, A.M., Kubo, R., Kappler, J.W., Marrack, P.: The Vb-specific superantigen staphylococcal enterotoxin B: Stimulation of mature T cells and clonal deletion in neonatal mice. Cell 56:27-36, 1989.

Claims

1. Pharmaceutical composition for treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens comprising a substance making said cells targets for cytotoxic T cells.

2. Composition according to claim 1 characterized in that said substance has the ability of activating cytotoxic T cells for inactivating or lysing said class II expressing cells.

3. Composition according to claim 1 or 2, characterized in that the substance is of bacterial origin and has the capability of interacting with particular sequences in the T-cell receptor V beta chains and directs T-cells to lyse the target MHC class II antigen expressing cell.

4. Composition according to claim 3 wherein the substance is selected from the group consisting of staphylococcal enterotoxines, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.

5. Composition according to claim 4, characterized by being chemical or genetical modifications of said structures.

6. Composition according to any of the preceding claims, characterized in that the substance is selected from the group Staphylococcal enterotoxin A, B, C, C_1, C_2, C_3, D and E, preferably Staphylococcal enterotoxin A.

7. Composition according to any of the preceding claims, characterized in that it comprises a combination of two or more enterotoxins, toxodis, active fragments or peptides thereof.

8. Composition according to any of the claims 1-7, characterized in that it is formulated as a parenteral composition.

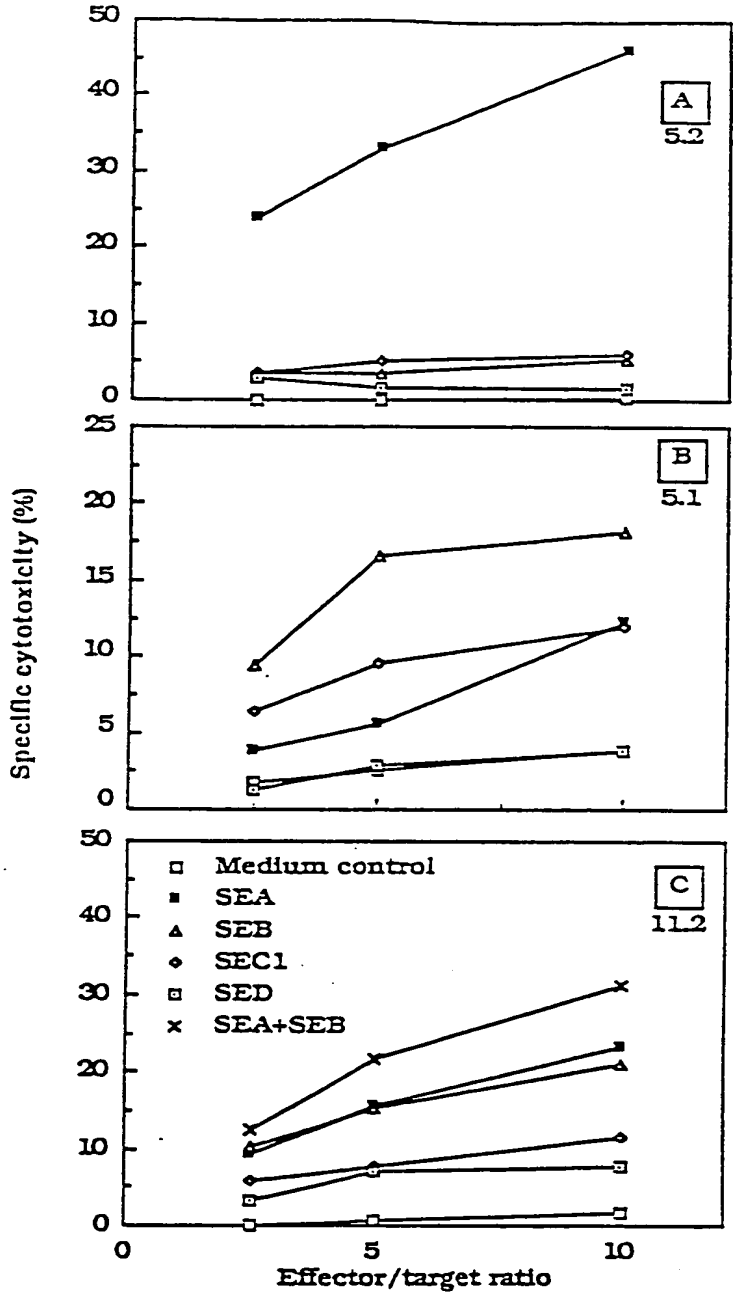
9. Composition according to claim 7, characterized in that it comprises parenterally acceptable vehicles, adjuvants and/or carriers.

10. Method of treating malignancies and autoimmune diseases involving cells expressing MHC class II antigens, comprising administering to a living body a composition including a substance making said cells targets for cytotoxic T cells.

11. Method according to claim 10, characterized in that the substance is selected from the group consisting of staphylococcal enterotoxines, toxoids, active fragments or peptides of said enterotoxins and substances having essentially the same mode of action, such as Toxic Shock Syndrome toxins (TSST-1), bacterial exoproteins, such as streptococcal exoproteins, and proteins produced by mycoplasma arthritidis, which have capacity to interact with parts of the TCR and specifically the V beta chains thereof.

12. Method according to claim 11, characterized in that the composition is administered parenterally.

13. Method according to claim 11, characterized in that the composition is administered systemically or locally.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5.2
B
5.1
C
11.2
Medium control
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SEB
SEC1
SED
SEA+SEB
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Effector/target ratio
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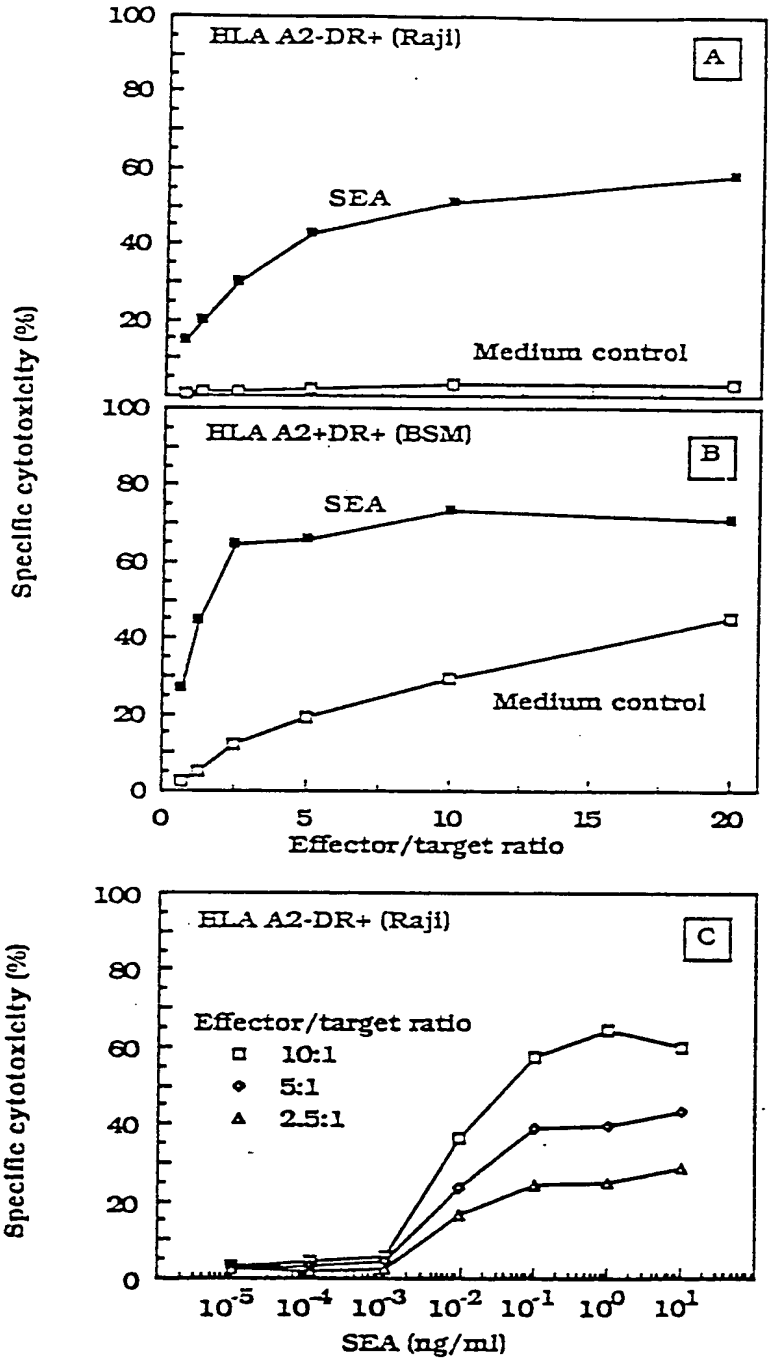
10
20
30
40
50
15
25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A
SEA
Medium control
HLA A2+DR+ (BSM)
B
SEA
Medium control
Specific cytotoxicity (%)
Effector/target ratio
HLA A2-DR+ (Raji)
C
Effector/target ratio
10:1
5:1
2.5:1
Specific cytotoxicity (%)
SEA (ng/ml)

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 90/00592

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: A 61 K 39/39, A 61 K35/74

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Nature, Vol. 339, May 1989 J D Fraser: "High-affinity binding of staphylococcal enterotoxins A and B to HLA-DR"", see page 221 - page 223 --	1-9
X	Dialog Information Services, File 154: Medline 83-90,NLM accession no. 89235137, P.M. Stuart et al. "Induction of class II MHC antigen expression in macrophages by Mycoplasma species.", & J Immunol May 1989, 142(10) p3392-3399 --	1-9
X	Dialog Information Service, File 154: Medline 83-90, NLM accession no. 89078460, M. Matthes et al: "Clonal analysis of human T cell activation by the Mycoplasma arthritidis mitogen (MAS)", & Eur J Immunol Nov 1988, 18 (11) p1733-1737 --	1-9

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
14th December 1990	1990 -12- 19
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Mikael G:son Bergstrand

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	WO, A1, 8909619 (ROTHMAN, ULF S E) 19 October 1989, see the whole document --	1-9
A	WO, A1, 8703487 (MCMICHAEL, JOHN) 18 June 1987, see the whole document --	1-9
A	EP, A2, 0223579 (NOVO INDUSTRI A/S) 27 May 1987, see the whole document --	1-9

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. PCT/SE 90/00592

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 90-11-28
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Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A1- 8909619	89-10-19	AU-D-	3420689	89-11-03
WO-A1- 8703487	87-06-18	EP-A-	0249629	87-12-23
		JP-T-	63501872	88-07-28
		US-A-	4689222	87-08-25
EP-A2- 0223579	87-05-27	AU-B-	598423	90-06-21
		AU-D-	6518486	87-05-21
		JP-A-	62126199	87-06-08
		US-A-	4816441	89-03-28